Listing of claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (currently amended). A mammalian *in vitro* mRNA decapping system comprising:

a) a ~~mammalian~~ polysome-free HeLa cell cytoplasmic extract ~~substantially free of polysomes~~;

b) a methylated cap analog; and

c) a cap-labeled mRNA substrate.

2. (currently amended). The mammalian *in vitro* mRNA decapping system of claim 1 wherein said ~~mammalian~~ HeLa cell cytoplasmic extract is ~~an~~ a HeLa S100 cell cytoplasmic extract ~~which comprises a 100,000 x g, 1 hour supernatant from a mammalian cell lysate~~.

3. (currently amended). The mammalian *in vitro* mRNA decapping system of claim 2 wherein said ~~mammalian~~ HeLa cell cytoplasmic extract is prepared by dialysis of said extract containing 10% glycerol.

this is clear.

4. (cancelled). The composition of claim 1 wherein said mammalian cell lysate is obtained from a mammalian cell or tissue.

5. (currently amended). The mammalian *in vitro* mRNA decapping system of claim ~~1~~ 2 wherein said ~~mammalian cell cytoplasmic extract is a~~ HeLa S100 cell cytoplasmic extract ~~which~~ comprises a 100,000 x g, 1 hour supernatant from a HeLa cell lysate.

6. (previously presented). The mammalian *in vitro* mRNA decapping system of claim 1 wherein said methylated cap analog is ^{7me}GpppG or ^{7me}GTP.

7. (cancelled). The composition of claim 1 comprising components for additionally detecting mRNA deadenylation and degradation.

8. (cancelled). The composition of claim 7 wherein said mammalian cell cytoplasmic extract is depleted of activity of proteins that bind polyadenylate.

9. (previously presented). The mammalian *in vitro* mRNA decapping system of claim 1 wherein said cap-labeled mRNA substrate is labeled at the alpha phosphate of the cap.

10. (previously presented). The mammalian *in vitro* mRNA decapping system of claim 1 wherein said cap-labeled mRNA substrate is labeled at the cap of said cap-labeled mRNA substrate by a label selected from the group consisting of a radioactive label, a non-radioactive isotopic label, a fluorescent moiety, a visibly-detectable moiety, a releasable substrate or a co-factor for a chemical and enzymatic reaction.

a co-factor for

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11. (previously presented). The mammalian *in vitro* mRNA decapping system of claim 1 wherein said cap-labeled mRNA substrate comprises poly(A) or at least one RNA element.

12. (previously presented). The mammalian *in vitro* mRNA decapping system of claim 11 wherein said RNA element is an AU-rich element.

13. (previously presented). The mammalian *in vitro* mRNA decapping system of claim 11 wherein said RNA element is a pyrimidine-rich element.

14. (cancelled). A polypeptide which has a molecular weight of about 50 to about 100 kilodaltons (kD) in molecular exclusion chromatography, precipitates with 20% ammonium sulfate, elutes at between about 440 to 500mM NaC1 from a heparin-Sepharose column, and decaps mammalian RNA.

15. (cancelled). A polynucleotide which encodes a polypeptide which has a molecular weight of about 50 to about 100 kilodaltons (kD) in molecular exclusion chromatography, precipitates with 20% ammonium sulfate, elutes at between about 440 to 500mM NaC1 from a heparin-Sepharose column, and decaps mammalian RNA.

16. (cancelled). An antibody which binds specifically and with high affinity to a polypeptide which has a molecular weight of about 50 to about 100 kilodaltons (kD) in molecular exclusion chromatography, precipitates with 20% ammonium sulfate, elutes at between about 440 to 500mM NaC1 from a heparin-Sepharose column, and decaps mammalian RNA.

17. (currently amended). A kit for measuring mRNA decapping *in vitro* comprising:

a) a ~~mammalian~~ <u>polysome-free HeLa</u> cell cytoplasmic extract;

b) a methylated cap analog; and

c) cap-labeled mRNA substrate.

18. (cancelled) The kit of claim 17 further comprising a cap-labeled mRNA substrate.

19. (previously presented). The kit of claim 17 wherein said cap-labeled mRNA substrate is labeled at the alpha phosphate of the cap.

20. (previously presented). The kit of claim 17 wherein said cap-labeled mRNA substrate is labeled at the cap of said cap-labeled mRNA substrate by a label selected from the group consisting of a radioactive label, a non-radioactive isotopic label, a fluorescent moiety, a visibly-detectable moiety, a releasable substrate or a co-factor for a chemical and enzymatic reaction.

21. (cancelled). The kit of claim 17 wherein said mammalian cell cytoplasmic extract is depleted of activity of proteins that bind polyadenylate.

22. (cancelled). A method for carrying out *in vitro* mammalian mRNA decapping comprising the steps of

a) providing the composition of claim 1,

b) incubating said composition at about 30°C for about 30 min and monitoring decapping by detection of release of label from said cap-labeled RNA.

23. (cancelled). A method for identifying a compound as a modulator of mammalian mRNA decapping comprising carrying out the method of claim 22 in the presence and absence of said compound, and correlating any change in decapping by the presence of said compound with modulator activity of said compound.

24. (cancelled). The method of claim 23 wherein said cap-labeled mRNA substrate comprises poly(A) or at least one RNA element.

25. (cancelled). The composition of claim 23 wherein said RNA element is an AU-rich element.

26. (cancelled). The composition of claim 23 wherein said RNA element is a pyrimidine-rich element.

27. (previously presented) The mammalian *in vitro* mRNA decapping system of claim 1 further comprising means for sequestering proteins that bind to poly(A).

+function (112, 6TH → OK)

28. (previously presented) The mammalian *in vitro* mRNA decapping system of claim 11 further comprising means for stimulating decapping of the cap-labeled mRNA substrate wherein the cap-labeled mRNA substrate comprises poly (A).

+function

29. (previously presented) The mammalian *in vitro* mRNA decapping system of claim 11 further comprising a cold poly(A) competitor RNA.

30. (previously presented) The mammalian *in vitro* mRNA decapping system of claim 12 further comprising means for reducing decapping of the cap-labeled mRNA substrate.

31. (currently amended) The mammalian *in vitro* mRNA decapping system of claim 12 further comprising an ~~ARE~~ AU-rich element competitor RNA.

32. (previously presented) The kit of claim 17 wherein the cap-labeled mRNA substrate comprises Poly(A).

33. (currently amended) The kit of claim ~~27~~ 32 further comprising means for stimulating decapping the cap-labeled mRNA substrate.

34. (currently amended) The kit of claim ~~27~~ 32 further comprising a cold poly(A) competitor RNA.

35. (previously presented) The kit of claim 17 wherein the cap-labeled mRNA substrate comprises an RNA element.

36. (currently amended) The kit of claim ~~30~~ 35 wherein the RNA element is an AU-rich element.

37. (currently amended) The kit of claim ~~31~~ 36 furthering comprising means for reducing decapping the cap-labeled mRNA substrate.

38. (currently amended The kit of claim ~~31~~ 36 further comprising an ~~ARE~~ AU-rich element competitor RNA.

39. (currently amended) A mammalian *in vitro* mRNA decapping system comprising:

a) a ~~mammalian~~ polysome-free HeLa cell cytoplasmic extract ~~substantially free of polysomes~~;

b) a cap-labeled mRNA substrate; and

c) + function means for decapping the cap-labeled mRNA substrate.

40. (currently amended). A kit for measuring mRNA decapping *in vitro* comprising:

a) a ~~mammalian~~ polysome-free HeLa cell cytoplasmic extract ~~substantially free of polysomes~~;

b) a cap-labeled mRNA substrate, and

c) + function means for decapping the cap-labeled mRNA substrate.

41. (new) The kit of claim 17 wherein the polysome-free HeLa cell cytoplasmic extract is a HeLa S100 cell cytoplasmic extract.

42. (new) The kit of claim 39 wherein the polysome-free HeLa cell cytoplasmic extract is a HeLa S100 cell cytoplasmic extract.

43. (new) The kit of claim 40 wherein the polysome-free HeLa cell cytoplasmic extract is a HeLa S100 cell cytoplasmic extract.